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Genetic Structure of Juvelin Grunt (*Pomadasys kaakan*) Using Amplified Fragment Length Polymorphism Technique in the Persian Gulf

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Abstract: Population genetic structure of Javelin Grunt, *Pomadasys kaakan* in the Persian Gulf was analyzed using amplified fragment length polymorphism (AFLP). For this purpose, six primer combinations were analyzed to study the genetic variation among 39 individuals in three populations (Abadan, Bushehr and Bandar Abbas) of the *P. kaakan*. In total, 88 of 410 putative loci were polymorphic (21.46%). The proportion of polymorphic loci and Nei's genetic distances for three populations ranged from 68.18% (Bandar Abbas) to 88.64% (Abadan) and from 0.0417 to 0.1121, respectively. Pairwise F_{ST} and average expected heterozygosity ranged from 0.0862 to 0.2756 and 0.222 to 0.330, respectively. In addition, UPGMA tree revealed significant geographic structure in this species. Pattern of isolation by distance was observed in this species, indicating that significant genetic differentiation among localities of *P. kaakan* was mainly due to the geographic distance.

Key words: Genetic Structure · Javelin Grunt · *Pomadasys kaakan* · Persian Gulf · AFLP

INTRODUCTION

The family of Haemulidae is one of the major commercially family through the Persian Gulf, which represented by three genus and ten species [1]. Among these species, Javelin Grunt, *Pomadasys kaakan* is an important commercial species that widely distributed across the Persian Gulf and Oman Sea [2]. This species is also distributed in Indo-West Pacific: Red Sea and east coast of Africa to Southeast Asia, north to Taiwan, south to Queensland in Australia [3]. This species mainly caught by Gillnets and Gargoor in the Persain Gulf [4]. Study on catch statistics of *P. kaakan* in Bushehr waters in the Persian Gulf showed a decrease from 1154 to 748 tons during 1998 to 2007 [5]. Al-Husaini et *al.* [6] also mentioned that the annual catch of this species from Kuwait have decreased from 318 tons in 1982 to 153 tons in 1999.

The decline in natural resources of a species can have negative impact on genetic diversity, which will be a serious problem for population of that species. Thus, assessment of population genetic structure makes up essential information for the purpose of fisheries management and resource recovery [7, 8]. Molecular markers are the useful methods to study of genetic variation and population structure in natural populations. To date, amplified fragment length polymorphism (AFLP) technique [9] has been utilized extensively in the field of population genetics. Showing the numerous polymorphic loci on a single polyacrylamide gel, high reproducibility as results of high PCR annealing temperatures and relative economy on a PCR marker basis are the major advantages of AFLP technique [10]. Moreover, this technique does not need any prior molecular information regarding sequences of the species under investigation [10]. Therefore, AFLP is

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especially applicable to study any species that the genome background is not well denoted, like *P. kaakan*. The objective of the present study was to determine the patterns of genetic structure among three separated *P. kaakan* populations in the Persian Gulf using AFLP technique.

MATERIALS AND METHODS

Three wild populations of *P. kaakan* were investigated from northern coasts of the Persian Gulf (Fig. 1). Samples were collected by local fisherman from Abadan (14 individuals), Bushehr (14 individuals) and Bandar Abbas (11 individuals). Then specimens immediately were preserved in 96% ethanol and transferred to the laboratory for DNA extraction and further analysis.

Total DNA was isolated followed by CTAB method described by Cota-sanchez e*t al*. [11]. Procedures of AFLP were essentially based on Vos *et al*. [9]. About 250 ng of genomic DNA for each specimen was digested with 10 units of *EcoRI* and *MseI* for 2 h at 37°C and then incubated for 2 h at 65°C. Ligation of adapters to digested temple DNA was carried out with T4 DNA Ligase at 22°C for 12 h. Five micro liters of 1:4 diluted ligation mixture were pre-amplified with primer E+A (5' GACTGCGTACCAATTC+A 3') and primer M+C (5' GATGAGTCCTGAGTAA+C 3') by running for 25 cycles at 94°C for 30 s, 56°C for 60 s and 72°C for 60 s. Then, 20 µL of PCR mixture product was diluted 5-fold with distilled water and used as templates for the subsequent selective PCR amplification. The selective amplifications were carried out in 20 µL PCR reaction volume on a gradient thermal cycler with a touchdown cycling profile of 12 cycles of 30 s at 94°C, 30 s at 65°C (decreasing with 1°C per cycle) and]60 s at 72°C followed by the cycling profile of 18 cycles of 30 s at 94°C, 30 s at 56°C and] 60 s at 72°C. The final step was a prolonged extension of 5 min at 72°C. Selective amplifications were performed using six primer pairs; (E-ACT/M-CAT, E-AGC/M-CCG, E-ACA/M-CAC, E-AAG/M-CAC, E-AGC/M-CAT and E-AAG/M-CCG). PCR products were run on 6.0% denaturing polyacrylamide gel electrophoresis (PAGE) at 80 W for 90 min and]finally detected using the silver staining method.

AFLP bands were scored for presence (1) or absence (0) of the bands and transformed into 0/1 binary character matrix. Proportion of polymorphic loci, Nei's genetic diversity and average heterozygosity were calculated by POPGENE software version 1.3. Pairwise genetic differentiation (*F*st) values between populations were computed with Arlequin software version 3.1. In addition,

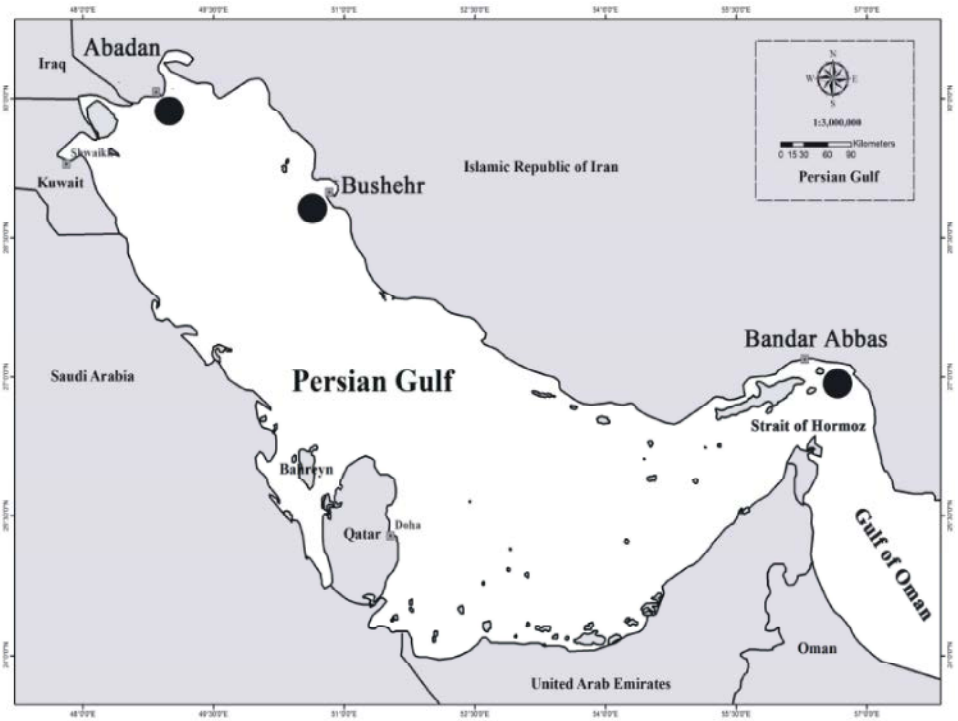

Fig. 1: Sampling locations of three populations of *P. kaakan* in northern coastline of the Persian Gulf

genetic relationships among populations were estimated by constructing UPGMA tree based on Nei's genetic distance [12] in TFPGA version 1.3. Analysis of molecular variance (AMOVA) and Principal coordinate analysis (PCA) based on Euclidian distances between AFLP multilocus phenotypes were performed using GENALEX software version 6.0.

RESULTS

Amplification results of six primer combination were shown in Table 1. In total, 410 bands were identified, which 88 bands were polymorphic (21.46%). The number of bands per primer combination and polymorphic bands obtained with each primer combination ranged from 49 to 90 and 6 to 19, respectively. The most abundant fragments were detected for the combinations E-ACA/ M-CAC and the least ones were E-AAG/ M-CAC.

Pairwise F_{ST} between populations and Nei's genetic distance were shown in Table 2. Pairwise F_{ST} values among populations were high and significant ($P< 0.05$), ranging from 0.0862 to 0.2756. Nei's genetic distance analysis also suggested that Abadan and Bandar Abbas populations were the most genetically different (0.1121), whereas the Abadan and Bushehr populations were the most similar groups (0.0417).

The population with the highest proportion of polymorphic loci (88.64) and highest number of polymorphic loci (78) was exhibited in Abadan population, whereas the lowest values were identified in Bandar Abbas population, in which the proportion of polymorphic loci and number of polymorphic loci were 68.18 and 63, respectively. Expected heterozygosity values varied from 0.222 to 0.330 (Table 3).

Table 1: Amplification results of six AFLP primer combinations

Primer combination	Number of amplified loci	Number of polymorphic amplified loci	Frequency of polymorphic loci
E-ACT/ M-CAT	70	12	17.14
E-AGC/ M-CCG	59	19	32.2
E-ACA/ M-CAC	90	18	20
E-AAG/ M-CAC	49	6	12.24
E-AGC/ M-CAT	62	15	24.19
E-AAG/ M-CCG	80	18	22.5
Total	410	88	21.46

Table 2: Pairwise F_{ST} (above) between populations and Nei's genetic distance (below)

Population	Abadan	Bushehr	Bandar Abbas
Abadan		0.0862	0.2756
Bushehr	0.0417		0.1150
Bandar Abbas	0.1121	0.0530	

Table 3: Population genetic parameters (mean ± SE)

Population	Number of polymorphic loci	Percentages of polymorphic loci	HE± SE
Abadan	78	88.64	0.330± 0.016
Bushehr	75	85.23	0.291± 0.017
Bandar Abbas	63	68.18	0.222± 0.020

HE, Expected heterozygosity; SE, Standard Error

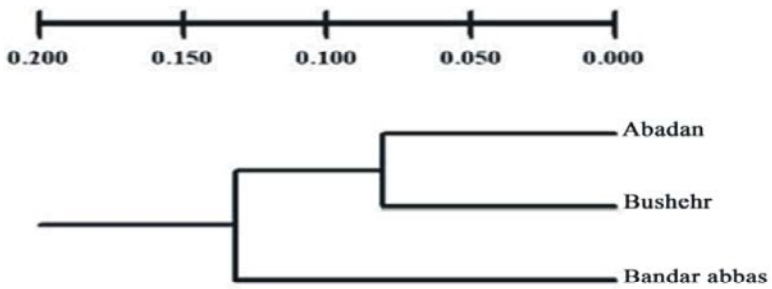

Fig. 2: UPGMA cluster analysis based on Nei's genetic distances among three populations

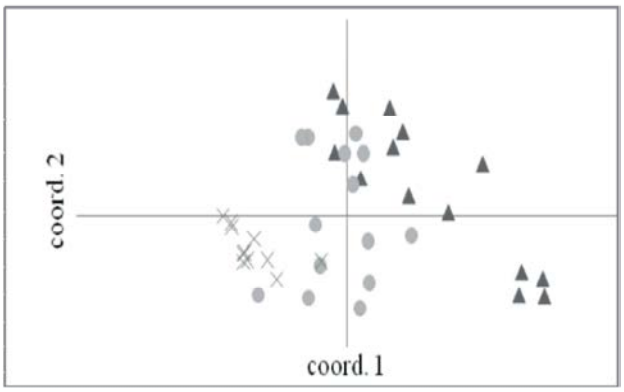

Fig. 3: Principal coordinate analysis based on Euclidian distances for 39 individuals of *P. kaakan* sampled from Abadan (▲); Bushehr (•) and Bandar Abbas (×)

Cluster analysis revealed two geographic groups; one group contains Abadan and Bushehr populations and another group was Bandar Abbas population (Fig. 2).

The plot of principal coordinate analysis (PCA) based on Euclidian distances provided a visual representation of genetic similarity between populations (Fig. 3). This plot revealed a clear isolation between Abadan and Bandar Abbas populations, whereas Bushehr population covered a part of other populations.

DISCUSSION

First molecular genetics background for *P. kaakan* populations was obtained by AFLP technique among three stations along northern coasts in the Persian Gulf. Results revealed a genetic differentiation among sample sites of the Persian Gulf.

The F_{ST} values among three sites were all significant ($P<0.05$), which indicates limited gene flow among populations. Also significant genetic differentiation was observed by Nei's genetic distance analysis between Bandar Abbas and other stations. Although, all populations were in the Persian Gulf, genetic differentiation was detected. Therefore, the hypothesis that *P. kaakan* in the Persian Gulf comprises a single stock was most probably rejected.

Geographic distance between Bandar Abbas and Bushehr populations is more than between Abadan and Bushehr populations; an explanation could be that the gene flow between Bandar Abbas population and other populations were limited by factors such as oceanographic characteristics and geographic distance. When dispersal of eggs or larvae is restricted, isolation by distances occurs within a population. For this species, results showed that eggs and larvae must be retained in spawning ground. Similar to our results, Pogson *et al.* [13] noted that one reason for observed genetic isolation in highly mobile Atlantic cod, *Gadus morhua*, was retention of eggs and larvae in the spawning ground. Other genetic structure research in the Persian Gulf on fish species like *Scomberomorus commerson* and *S. guttatus* raveled a genetic connectivity between sampled sites due to highly migratory vagile species with planktonic larvae such as members of the genus Scomberomorus [14, 15].

Liu *et al*. [16] mentioned that many marine organisms have pelagic larvae that can potentially interconnect far off populations through dispersal on ocean currents. The water circulation in the Persian Gulf consists of inflow from surface of Strait of Hormuz to the Persian Gulf (along the southern coasts of Iran) and outflow of water (along the coasts of Arabian country) from bottom of Strait of Hormuz [17]. Therefore, the water circulation in this region may not be responsible for the significance of genetic differentiation in *P. kaakan* populations.

The UPGMA tree indicated that the three studied *P. kaakan* populations were divided into two groups. Geographic distances could increase genetic distances between *P. kaakan* populations, in agreement with an isolation by distance model that proposed by Kimura and Weiss [18], which expressed gene flow in which migration only occurs among neighboring demes. Nei's genetic distance analysis also showed that genetic distance between Bushehr and Bandar Abbas is larger than genetic distance between Abadan and Bushehr. This may correlated with geographic distance between these stations.

It is concluded that gene pool of *P. kaakan* in the Persian Gulf isn't homogeneous, due to local condition, geographic distances and species life history. Results of this study can be important for fisheries management of this species in the Persian Gulf in the future, but the boundaries of the populations remain unknown.

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